NOWOTWORY Journal of Oncology • 2002 • volume 52
Number 1 • 16–23

Original contributions

Primary mediastinal large B-cell lymphoma. Lymphoma in many ways unlike other large B-cell lymphomas*

Renata K. Maryniak, Katarzyna Roszkowska-Purska

Introduction. Mediastinal large B-cell lymphoma (PMBL) is a rare and not easily recognized entity. Histopathological diagnosis is difficult without immunohistochemical studies, due to high cells pleomorphism.
Aim. Evaluation of sections from 51 lymphomas, selection of the panel of antibodies useful in differential diagnosis and attempt at determination of clinical and morphological prognostic factors.
Material and methods. 51 cases of PMBL diagnosed and treated at the Institute of Oncology in Warsaw. Clinical symptoms and varied histopathological appearences with immunohistochemical studies were presented. Statistic analysis of selected prognostic parameters was performed.
Results. Morphologically the lymphoma resembled several other tumours with mediastinal localization and CD20 staining was decisive for correct diagnosis. Tumour size had a statistically significant effect on overall survival. Among other analyzed parameters only a few showed a tendency for correlation with survival.
Conclusions. PMBL is a subtype of DLBCL with distinct clinical presentation and histopathological features requiring differential diagnosis with other mediastinal tumours.

Pierwotny chłoniak śródpiersia z dużych komórek B. Chłoniak odmienny od innych chłoniaków z dużych komórek B

Wstęp. Pierwotny chłoniak śródpiersia z dużych komórek B jest rzadką jednostką, histopatologicznie trudną do rozpoznania bez badań immunohistochemicznych ze względu na znaczny pleomorfizm utkania.
Materiał i metody. Analiza dotyczyła 51 chorych z PMBL. Dane kliniczne zebrano z historii chorób. Oceniono morfologicznie i immunohistochemicznie wycinki z 51 guzów śródpiersia oraz przeprowadzono analizę statystyczną wpływu wybranych parametrów klinicznych i histopatologicznych na przeżycie chorych.
Wyniki. Ze względu na znaczny pleomorfizm komórek wyodrębniono 5 podtypów utkania. Ze względu na podobieństwo do innych nowotworów w tej lokalizacji niezbędne jest wykonanie barwienia immunohistochemicznego z CD 20. U 21 pacjentów uzyskano CR z czasami przeżycia całkowitego od 36 do 99 miesięcy. Żyje 24, wśród nich 3 z przetrwałą chorobą. Pozostałych 26 zmarło pomimo stosowanego leczenia, z utrzymującym się guzem i progresją.
Wnioski. PMBL to chłoniak morfologicznie bardzo zróżnicowany. Dla prawidłowego rozpoznania konieczne jest immunohistochemiczne wykazanie ekspresji CD 20. Analiza statystyczna wykazała, że wielkość guza ma istotny wpływ na całkowite przeżycie. Inne badane parametry wykazały jedynie tendencję do krótszych przeżyć.

Key words: Mediastinal large B-cell lymphoma, varied histology, prognostic factors
Słowa kluczowe: chłoniak śródpiersia z dużych komórek B, różnorodność utkania, czynniki prognostyczne

Introduction

The mediastinum may be the primary localization of various tumours: thymoma, thymic carcinoma, benign and malignant soft tissue tumours [1] and germinal tumours with the most frequent, i.e. seminoma. Nodular sclerosis Hodgkin lymphoma commonly presents in the mediastinum as well as some 15-25 % of non-Hodgkin lymphomas [2-4]. Precursor T-lymphoblastic lymphoma is the most frequent, but sporadically one may encounter anaplastic (CD30-positive) large cell lymphoma [5]. Tumours from other organs may metastasize to the mediastinum, among them squamous or small cell anaplastic or undifferentiated pulmonary carcinoma, melanoma, renal clear cell carcinoma and others [1].

Primary mediastinal large B-cell lymphoma (PMBL) has been described fairly recently. Hodgkin lymphoma

Laboratory of Pathomorphology,
Institute of Haematology, Warsaw,
Department of Pathology,
The Maria Skłodowska-Curie Memorial Cancer Center
and Institute of Oncology, Warsaw, Poland

* Based on Ph.D. thesis of Dr K. Roszkowska-Purska

was the first to be recognized among lymphoid mediastinal tumours. Development of immunocytochemical methods resulted in rapid progress in diagnosis and classification of lymphomas [6]. In the late 1970ies T-lymphoblastic lymphoma presenting in the mediastinum had been morphologically and immunologically defined [7-9]. In the early 1980ies several authors noted non-Hodgkin, non-lymphoblastic, mediastinal large cell lymphomas associated with characteristic clinical symptoms [10-13]. The disease mostly affected young women in the third or fourth decade of life and manifested as anterior mediastinal tumour, frequently with the superior vena cava syndrome, generally without involvement of other localizations. The lymphoma was finally defined as B-cell type [5, 14, 15]. Heterogeneity of these mediastinal tumours was observed and authors warned against diagnosing them merely on morphological grounds [1, 3, 10, 14, 16]. Very often it was impossible to classify mediastinal large cell lymphomas according to any of the existing classifications of malignant lymphomas [6, 17]. Lamarre et al. [16] introduced the term „primary mediastinal large B--cell lymphoma". Their study of 29 cases of PMBL included 18 women and 11 men with median age of 32 years. The patients presented with the superior vena cava syndrome and had localized disease (Ann Arbor stage I or II) at diagnosis. General symptoms were present in about 1/3 of the cases. Morphologically, the tumour cells displayed great pleomorphism. The diagnosis of B-cell lymphoma was made owing to positive result of staining with anti-CD 20 antibody and lack of reactivity with anti--pan T antibody. Similary to Jacobson [18], prognosis better than previously reported was observed, due to introduction of aggressive chemotherapy combined with radiotherapy.

Primary mediastinal large B-cell lymphoma (PMBL) with a characteristic clinical presentation had been defined as a histoclinical entity acc. to the REAL classification [19]. Although acc. to the WHO classification it is a subtype of diffuse large B-cell lymphoma (DLBCL), it is separately discussed as „mediastinal (thymic) large B-cell lymphoma" with distinctive clinical, immunophenotypic and genetic features [20]. PMBL is a rare lymphoma and it constitutes 0.9 to 3,7% of all non--Hodgkin lymphomas and 2,4 to 6.5% of large cell lymphomas [10, 18, 21, 22, 35]. It affects young people (median age 32 years), predominantly women [14, 16, 23-28] and presents on routine X-ray examination or CT of the chest as an anterior mediastinal mass. PMBL invades the adjacent anatomic sites by continuity, i.e. large vessels, pleura, pericardium, trachea and the anterior chest wall. The most frequent symptom is the superior vena cava syndrome. Although a few symptomless cases have been reported, the majority of patients complain of pain or discomfort in the chest, dyspnea or cough [14, 22, 24, 27]. Some patients have B-symptoms at diagnosis. The majority of patients are clinically in Ann Arbor stage I or II [29]. When the disease disseminates outside the thorax, it usually involves the supraclavicular, cervical and axillary lymph nodes. Only sporadically primary involvement of other organs (kidneys, adrenal glands, liver or spleen) or retroperitonal lymph nodes, had been reported [3, 14, 24]. In comparison with other large B-cell lymphomas, bone marrow involvement at diagnosis is very seldom noted in PMBL [24]. Morphologically primary mediastinal large B-cell lymphoma displays significant pleomorphism [3, 25] and some degree of sclerosis is a common feature [20]. Patients with PMBL are treated with combination chemotherapy followed by radiotherapy [16, 30]. PMBL belongs to a group of highly malignant lymphomas with an aggressive, life threatening clinical course. It is important to consider prognostic factors at diagnosis. Clinical factors defined by International Prognostic Index [31] are: age, Ann Arbor clinical stage (I-IV), performance status (0-5), serum LDH level and number of extranodal localisations. Evaluation of these parameters enables to predict tumour growth rate, its invasive potential, physical condition of the patient and tolerance of intensive therapy. Poor prognosis is related to large primary tumour (over 10 cm in diameter), lack of remission after the first treatment or to cases of rapid recurrence. Recurrences in other localizations, besides the mediastinum, carry particularly adverse prognosis [18, 24, 32]. Studies from the 1980ies stressed the adverse association of prognosis to the lack of response to treatment. At present, with aggressive chemotherapy and radiotherapy, complete remissions are more frequently achieved, and in more than half of the treated patients 5--year survival is observed [18, 24, 30, 32]. Patients who do not achieve remission or in whom it is very short and followed by progression in the mediastinum or at distant sites, usually die within the first two years. Besides the clinical, histopathological prognostic factors have been searched for and the tumour cell proliferation rate (Ki-67 index) has been one of the earliest and still considered [33]. It is assumed that high values of this index (60-80% and over) correlate with longer overall survival in treated patients [34, 35]. On the other hand in untreated patients (retrospective studies), high values of the proliferative index correlated with shorter survival. Gascoyne [36] and Wilson et al. [37] suggest that lymphomas with a low proliferation index show lower sensitivity to treatment in comparison to those characterized by high proliferation index.

Expression of bcl-2 protein is an independent prognostic factor [31] and it does not correlate with the presence of the bcl-2 gene rearrangement. While the presence of t(14;18) accompanied by rearrangement of the *bcl-2* gene is observed in 15-25 % large cell lymphomas, the bcl-2 protein expression is noted in about 25-50% of these lymphomas [38]. It means that there must be some other mechanism, besides the translocation, which would be responsible for high expression of bcl-2 protein in those lymphomas [36]. The bcl-2 protein is a biological factor blocking apoptosis of the cells. Anti-apoptotic effect of the bcl-2 protein may alter the expected results of chemo- or radiotherapy, therapeutic methods inducing neoplastic cell death. Therefore, large B-cell lymphomas characterized by high expression of bcl-2 protein

may manifest resistance to chemotherapy or radiotherapy, resulting in lack of response to treatment.

Material and methods

Clinical material

The study was held on 51 patients with primary mediastinal large B-cell lymphoma diagnosed in the Department of Pathology, of The Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology in Warsaw in the years 1991-1998. The group consisted of 28 women and 22 men aged from 17 to 67 years, (mean age 31,9 years). There was a predominance of women to men of 1.3: 1. Female patient age was 17-68 years (mean: 31.5), male patient age – 17 – 59 years (mean: 32.3). Clinical diagnosis of mediastinal tumour was made in all 51 patients on radiological and/or CT examination of the chest. The size of the tumour was measured in centimeters, and when the diameter exceeded 10 cm, it was considered to be large. The extent of the disease was evaluated by physical examination, USG or CT of the chest and the abdomen and, in 49 patients, by bone marrow biopsy. Patients were staged according to the Ann Arbor system [39]. Serum lactate dehydrogenase (LDH) levels were determined in clinical laboratory with the maximum normal value 300 IU/l. Patients were treated with chemotherapy according to the regimens: CHOP or MEVA (V-VIII courses) followed by radiotherapy of the mediastinum (3600 – 4200 cGy/t). Results of the treatment were expressed as complete remission (CR) or no remission (NR) and as overall survival (OS) in months. Complete remission was calculated from the moment of clinical observation of disease regression following therapy till the moment of the last observation.

Histopathological material and methods

Biopsies from mediastinal tumours were obtained by mediastinoscopy or thoracotomy in 34 patients, from the lymph nodes (bronchiothoracic, supraclavicular and cervical) in 10 patients, from pleural and anterior chest infiltrations in 6 patients and from the superior part of the tumour above the clavicula in 1 patient. Tissue samples were routinely processed and parafin section were hematoxylin and eosin stained. Lymphomas were diagnosed according to the REAL classification. Additional paraffin sections were prepared for immunohistochemical studies. A panel of antibodies was used: LCA, CK-MNF, CD 20, CD 3, CD15, CD 30, kappa, lambda, MIB1 and bcl-2. However, not in all the cases tissue samples were large enough to perform all the immunocytochemical staining.

Statistic methods

Only 50 patients were subject to the analysis (one patient was excluded due to incomplete clinical data). Follow-up was conducted from January 1991 till March 2001, that is in time brackets from 9 to 2 years. A two-year follow-up was the shortest and concerned patients in whom the diagnosis was made in the years 1991-1998.

Statistic analysis included:

- Preparation of distribution of the analysed quantitative parameters (sex, stage, response to treatment, performance status at last follow up) and presentation in groups of quantitive parameters (age, tumour size, LDH level, MIB1 expression, bcl-2 protein expression and overall survival) in numerical values and as percentage of all the studied patients.
- Analysis of the effect of the above mentioned parameters on duration of CR or NR – chi2 test.
- Analysis of survival
  a) traditional method of Life Tables
  b) comparison of curves – function of survival by log-rank test

Results and discussion

Clinical

Basic data and clinical results are presented in Table I. In 21 patients the presence of the superior vena cava syndrome was the main clinical symptom. Most of the patients complained of pain or discomfort in the chest, cough and dyspnea. Recurrent infections of the upper respiratory tract were manifestations of the tumour in 4 patients. Two patients had no complaints and the mediastinal tumour was accidentally found on the radiological examination of the chest. General symptoms were present from the beginning of the disease in 23 patients. Aggressive chemotherapy and radiotherapy of the mediastinum were given. Complete remission was achieved in 21 patients with overall survival ranging from 36 to 99 months. 24 patients are alive, of whom 3 live with persisting disease. In the remaining 26 patients the treatment did not induce regression and they died due to persistence of mediastinal tumour or progression to distant localisations. One patient was lost to follow-up.

Histopathological

Morphological examination of tissue sections disclosed significant pleomorphism of tumour cells. A range of morphologic appearances facilitated allocation of cases to five morphological groups. There were lymphomas composed of germinal center cells (centroblasts and centrocytes), of „clear" cells, polymorphous large cells, some contained cells resembling Reed-Sternberg and Hodgkin cells and some were small cell proliferations. Most lymphomas displayed high or medium mitotic activity. Various forms of sclerosis and/or hyalinisation of the stroma were present in a majority of tumours. In all cases immunohistochemical studies confirmed the diagnosis of large B-cell lymphoma with fenotype: LCA+, CD20+ and lack of expression of cytokeratin, CD3, CD30 and CD15. Only in 3 out of the 45 stained sections monoclonal immunoglobulin light chain production was detected. On the basis of routinely stained HE sections, it may be often impossible to make a correct diagnosis. Not only due to morphological pleomorphism of lymphoma, mimicking other mediastinal tumours, but often due to minute size of the sections, displaying frequently the so-called „crush" artefact caused by tissue damage during surgical excision.

In 45 out of 51 cases immunohistochemical reaction with MIB1 antibody (Ki-67) was performed to evaluate the proliferative activity of lymphoma cells. Depending on the percentage of lymphoma cells expressing MIB1 antigen, the cases were divided into groups:

80-100% cells expressing MIB1 – 20 cases
50 – 80% cells expressing MIB1 – 13 cases
<50% cells expressing MIB1 – 12 cases

Bcl-2 protein expression was also immunohistochemically tested in 45 cases. Only in 28 cases cytoplasmic labelling was noted and these were subdivided into 2 groups depen-

Table I. Basic clinical data and results in 51 patients with PMBL

No	Age	Sex	Tumour size (cm)	Ann Arbor clinical stage	LDH (IU/l)	CR/NR (mth)	OS (mth)	Deaths
1	52	M	>10	IA	-	NR	13	+
2	46	F	>10	IA	239	CR87	99	
3	30	F	>10	IB	-	-	1	+
4	36	F	-	-	-	-	-	-
5	25	F	>10	IVB	-	NR	7	+
6	45	M	>10	IB	-	NR	15	+
7	43	F	>10	IIA	686	NR	24	+
8	33	F	<10	IA	-	NR	11	+
9	35	M	<10	IA	345	CR-82	88	
10	17	M	<10	IA	319	CR-68	74	
11	19	F	>10	IA	480	CR-68	74	
12	47	F	>10	IIB	1532	NR	24	+
13	17	F	-	-	-	NR	10	+
14	17	F	>10	IIB	1003	NR	12	+
15	17	F	>10	IA	1074	NR	2	+
16	48	M	>10	IA	-	CR-74	80	
17	32	M	<10	IA	227	NR	8	+
18	31	F	>10	IA	1010	NR	15	+
19	19	F	>10	IIB	2107	NR	1	+
20	18	F	10	IA	1360	CR-52	60	
21	22	M	-	-	-	NR	4	+
22	59	M	>10	IVB	355	NR	10	+
23	19	F	>10	IIIA	1092	NR	37	+
24	41	F	>10	IA	525	NR	12	+
25	39	M	<10	IIB	651	CR-46	52	
26	22	M	>10	IIIB	644	NR	8	+
27	31	F	-	IA	-	CR-45	51	
28	40	F	<10	IA	260	CR-48	53	
29	33	F	>10	IB	218	CR-50	56	
30	28	M	>10	IB	1829	CR-32	39	
31	17	F	>10	IIEB	1051	NR	12	+
32	28	M	-	-	-	CR-?	43	
33	31	F	<10	IIB	884	NR	13	+
34	19	F	>10	IIB	408	CR-34	42	
35	18	F	>10	IIA	85	CR-28	36	
36	23	M	>10	IB	302	NR	9	+
37	21	M	>10	IA	417	CR-25	40	
38	25	M	>10	IIB	1919	NR	21	+
39	20	M	<10	IA	815	CR-40	45	
40	43	M	<10	IB	544	CR-38	45	
41	20	M	<10	IA	290	CR-38	46	
42	18	M	>10	IVB	3547	NR	18	+
43	38	F	<10	IB	250	CR-21	36	
44	32	F	>10	IIA	1160	CR-30	36	
45	50	F	>10	IIB	529	NR	29	
46	68	F	<10	IA	1915	NR	12	+
47	34	F	>10	IB	956	NR	8	+
48	29	M	>10	IB	850	CR-?	52	
49	27	F	<10	IA	-	NR	24	
50	40	F	>10	IA	681	NR	24	
51	25	M	>10	IIB	705	NR	1	+

LDH – serum lactate dehydrogenase level
CR – complete remission (in months)
NR – no remission (in months)
OS – overall survival (in months)

ding on the percentage of cells displaying bcl-2 protein expression;
>50% cells – 22 cases
< or = 30% cells – 6 cases
In the 17 remaining cases lymphoma cells did not display the expression of bcl-2 protein.

Statistic results

Distribution of the analyzed clinical and histopathological parameters is presented in Table II.

In an analysis of the effect of several parameters on the results of treatment, that is obtaining either complete remission (CR) or no remission (NR), it was disclosed with the chi2 test, that the tumour size had a statistical significant effect, as shown in Table III.

Table II. Distribution of the analyzed clinical and histopathological parameters

Parameter	Number of cases	%
Sex		
females	29	43
men	22	57
Age		
up to 39 years	37	72
40 years and more	14	28
Tumour size		
<10 cm	14	30
>10 cm	32	70
no data	5	
Ann Arbor clinical stage		
I i II	42	89
III i IV	5	11
no data	4	
LDH		
to 300 IU/l	6	15
301 – 1000 IU/l	20	51
above 1000 IU/l	13	34
no data	12	
MIB 1		
to 50%	18	40
above 50%	27	60
no data	7	
bcl-2 protein		
0	17	38
1-30 %	6	13
above 30%	22	49
no data	7	

Analysis of other clinical and histopathological parameters did not show any statistically significant differences between the studied groups. In an analysis of overall survival (OS) calculated with the traditional method of Life Tables, for the entire group of patients, the percentage of 2 year survival was 54%, at standard error 7.0, and 5 year survival (for patients diagnosed in years 1991-1995) – 27.3% with standard error 9.5. In analysis of the ratio of 2 year survival depending on tumour size, a tendency for shorter survival was noted in the group with tumour diameter over 10 cm (Table IV).

In an analysis employing the traditional method of Life Tables in relation to other clinical and histological parameters, no statistically significant differences were found regarding 2 year survival. Two year survival curves were also plotted using the Kaplan and Meier method and survival curves were compared between the categories of the analyzed parameters with the use of log-rank test (Table V).

Discussion

The above discussed group of 51 PMBL is not very numerous, but this unique lymphoma is rare. The largest study published so far was based on 141 cases [24], while the few other reports [3, 16, 23-25, 28, 29, 40] presented some 10 to 20 cases. Altogether over 300 cases of PMBL have been reported. This study further confirms several of the

Table III. Response to treatment of patients with primary mediastinal lymphoma depending on tumour size

Tumour size	Response					
	Complete remission - CR		No remission – NR		Total	
	Number of patients	%	Number of patients	%	Number of patients	%
<10 cm	9	64.3	5	35.7	14	100.0
>10 cm	10	32.3	21	67.7	32	100.0
Total	19	42.2	26	57.8	45	100.0

Table IV. Overall 2 year survival of patients with primary mediastinal lymphoma depending on tumour size

Survival in years	Tumour size			
	<10 cm (n=14)		>10 cm (n=32)	
	Survival rate %	Standard error	Survival rate %	Standard error
1 year	85.7	9.4	71.9	7.9
2 years	71.4	12.1	46.9	8.8

n – number of patients p=0.065

Table V. Comparison of survival curves between the categories of analyzed parameters with log-rank test

Parameter	Probablility p	Interpretation of the results
sex	0. 462	non significant survival curves
age	0. 896	non significant survival curves
tumour size	0. 0436	significantly shorter survival of patients with tumours >10 cm
Ann Arbor clinical stage	0. 110	tendency for shorter survival of patients with stage III and IV
LDH level	0. 166	tendency for shorter survival of patients with higher LDH level
MIB1 (<50% and >50%)	0. 807	non significant survival curves
MIB1 (<80% and >80%)	0. 834	non significant survival curves
bcl-2 (0%, 1-30% and >30%)	0. 168	tendency for shorter survival of patients with bcl-2 = 0%

characteristic features of PMBL. Clinically there was a predominance of women to men (1.3: 1), and 71.4% were young women (aged 17-39 years), which is considered very characteristic for this entity [3, 14, 16, 24, 27]. The majority of our 42 out of 51 patients (82.4%) presented in Ann Arbor stage I or II, and CR was achived in this group in 87.9% of patients. On the contrary, no response to treatment (NR) was observed in patients in Ann Arbor stage III and IV; they all died. Cazals et al. [24] considered their patients in Ann Arbor stage I and II (68% of patients) as a good prognosis group, in comparison to other diffuse large B-cell lymphomas at the same (early) clinical stage of involvement. Bone marrow involvement in the presented study was found only in 2 patients (4.2%). Cazals et al. [24] noted that bone marrow involvement in their 2% cases was significantly less frequent than in DLBCL. Other authors [3, 23, 41] stressed the lack of bone marrow involvement. General symptoms are uncommon in PMBL, but when present, they indicate poor prognosis [40]. In our material they were present in 23 (45.1%) of patients. In 14 of those no remission was achieved following the treatment and they died, with overall survival from 1 to 24 months. Among other clinical features of prognostic significance are: the size of primary tumour and LDH level at diagnosis. In 32 (69.6%) of our patients mediastinal tumour was over 10 cm in diameter and CR was obtained only in 10 of them (32.3%). The remaining patients had no remission and all but one died. Of 14 cases of tumours below 10 cm in diameter CR was achieved in 9 (Table III). Statistical analysis showed correlation between the size of the primary tumour and the duration of overall survival ($p=0.0436$) with significantly shorter survival of patients with tumour size >10cm. Jacobson et al. [18] and others [3, 27, 31] reported similar findings stressing that tumour size was an important prognostic factor correlating with the achievement of CR following the treatment. Regarding serum LDH level, in 33 of our patients it was elevated and among those in 13 (33.3%) highly elevated – 10 of them died with NR. Among 6 patients with normal LDH level CR was achieved in 5. Statistical analysis of this parameter showed a tendency for shorter survival of patients with an elevated LDH ($p>0.166$). The opinions in literature regarding the prognostic significance of LDH are different. Jacobson et al. [16] did not observe any differences in survival between patients with high and normal LDH levels. Others [5] showed that although high LDH levels are more frequent in PMBL (76%) than in other large B cell lymphomas (51%), no correlation was found between LDH levels and effects of treatment in PMBL. However our material is too small for valid statistic analysis due to PMBL rarity.

On histopathological examination of PMBLs, the cells may appear uniform or display significant pleomorphism resembling a variety of other neoplasms that may be also present in the mediastinum. A frequently described „clear cell pattern" of PMBL [15] may be erroneously interpreted as mediastinal seminoma or metastatic renal cell carcinoma. Many authors called attention to primary misdiagnoses of Hodgkin lymphoma, thymic carcinoma or even sarcoma in their cases of PMBL [3, 14, 41]. Immunohistochemical staining with CD20 has been established as absolutely indispensible in the diagnosis of mediastinal tumours. When tissue samples are very small and with crush artifact resembling small cell lung cancer and routinely stained HE sections are very difficult to interpret, immunocytochemical staining with cytokeratin and CD 20 will differentiate between carcinoma and B-cell lymphoma. Due to aggressive tumour growth and a tendency to infiltrate the adjacent anatomic sites it is very important to establish the diagnosis without any delay and implement the treatment. Therefore, the statement of Lamarre and collegues [16] that: „The single most important factor in establishing the correct diagnosis is a high index of suspicion on the part of the pathologist" is not outdated.

At present oncological diagnostics are frequently based on cytology and one must be aware that it is not sufficient in lymphomas. In 2 of our cases, the first diagnostic material was a FNAB of mediastinal tumour where on the basis of cytological appearance squamous cell carcinoma was diagnosed in one case and an undifferentiated carcinoma in the other. Correct diagnosis was not established until later, when a tumour biopsy was taken and immunohistochemical studies enabled the diagnosis of large B-cell lymphoma. Cytopathological reports [42] of the last few years warn that FNAB of mediastinal tumours is of diagnostic value only when supplemented by immunohistochemical studies. Slagel et al. [43] mentioned the possibility of incorrect diagnosis in cytological material of various mediastinal tumours which contain a spindle cell component. Yu [44] presents a case of thymic epithelium entrapped by PMBL, which happened to be the main cytological component causing misdiagnosis of thymoma. It is apparent that in case of mediastinal tumours, histopathological evaluation of tissue sections supplemented by immunohistochemical studies is the most sensitive diagnostic procedure. Rarely, a supraclavicular or cervical lymph node is the primary diagnostic material. However with the knowledge of the characteristic clinical presentation of this mediastinal tumour and with the aid of immunohistochemical staining the pathologist will be able to make a correct diagnosis of PMBL.

Searching for morphological prognostic factors, the cell proliferation index was evaluated in 51 cases. Slightly better effects of treatment were observed in cases with MIB1>50% (out of 21 patients with CR, in 11 MIB1>50%, and in 8 it was between 80-100%). This observation would be in agreement with data from literature [24, 25, 34, 35, 37].

Evaluation of bcl-2 protein expression in lymphoma cells is also a morphological prognostic factor. High expression usually correlates with diminished sensitivity to treatment, while lack or low expression is associated with great sensitivity to treatment. In our study, high and medium bcl-2 protein expression was found in 22 cases – 12 of them did not achieve remission, 10 died subsequently. In 23 cases bcl-2 protein was not expressed or expressed in less than 30% of cells. Only 9 patients of this group

achieved CR following the treatment. Our observation differs from that of other authors, but it may require further studies on a larger material to clarify this point.

Regarding histogenesis, PMBL derives from thymic B-cells [14, 20]. Its immunophenotype is identical with that of these cells (CD19 and CD20+, CD21, CD5 and CD10-). The results of the above presented study as well as those from literature indicate that cytoplasmic or surface immunoglobulin expression is seldom found [14, 20], although genetic studies show rearrangement of the heavy and light chain immunoglobulin genes [20]. Molecular studies on 3 translocations common in B-cell non-Hodgkin lymphomas indicate essential differences between PMBLs and other DLBCLs [45, 46]. Two of these chromosomal abnormalities, t(14;18) involving bcl-2 gene and t(8;14) involving c-myc gene, show strong association with histological subtypes of lymphomas. The first one is common in follicular lymphomas, and in about 30% of the DLBCLs, while the second one appears in Burkitt's and Burkitt type lymphomas, in AIDS-associated lymphomas and in about 20% of DLBCL [45]. The bcl-6 gene is expressed in non-Hodgkin lymphomas originating from germinal center B lymphocytes and it is associated with chromosomal abnormalities frequently found in DLBCLs [46]. The studies of Scarpa et al. [45] on a series of 6 and later Tsang et al. [46] on 16 PMBLs showed a variety of molecular changes; however, bcl-2 gene rearrangement had not been detected in any of the 2 sets of cases. Only in one of the 16 PMBL cases [46] the bcl-6 gene rearrangement was found. The c-myc abnormalities were detected in 3 plus 3 cases from each study. Copie-Bergman has recently reported [47] the presence of MAL gene in 8 out of 12 cases of PMBL and MAL protein expression in 7 out 12. Just to compare, 8 control cases of DLBCL were analyzed and only in 2 low levels of MAL m-RNA were detected but with no protein expression. Genetic analysis of 26 PMBL cases [48] disclosed several characteristic abnormalities, mainly gains concerning chromosomes 2, 12, X and 9 and amplification of the REL gene. Among these, chromosome 9 overrepresentation was considered quite unique since it does not appear in any other non-Hodgkin lymphoma.

All the new findings mentioned above indicate that PMBLs display molecular and genetic changes that differ from those characteristic for other DLBCLs. Thus it is fully justified to define primary mediastinal large B-cell lymphoma as an entity with distinct clinicopathological and biological features.

Conclusions

1. In the analyzed group of 51 cases of PMBL a great variety of morphological pattern was noted. Some lymphomas resembled other neoplasms that may be localized in the mediastinum.
2. In histopathological evaluation of mediastinal tumours it is indispensible to do a panel of immunohistochemical stains enabling differential diagnosis between epithelial tumours, Hodgkin lymphoma and T or B lymphomas. It is equally important to be familiar with clinical presentation of the tumour in each case.
3. Statistic analysis showed that tumour size had a statistically significant effect on overall survival. Other analyzed parameters only displayed tendency for correlation.

Doc. dr hab. med. Renata K. Maryniak
Laboratory of Pathomorphology
Institute of Haematology
Chocimska 5, 00-957 Warsaw, Poland

References

1. Suster S, Moran CA, Koss MN. Rhabdomyosarcoma of the anterior mediastimun: report of four cases unassociated with germ cell, teratomas or thymic carcinomatous components. *Hum Pathol* 1994; 25; 349-356.
2. Benjamin SP, McCormack LI, Effler BB, Groves LK. Primary lymphatic tumors of the mediastinum. *Cancer* 1972; 30: 708-712.
3. Menestrina F, Chilosi M, Bonetti F et al. Mediastinal large-cell lymphoma of B-type with sclerosis: histopathological and immunohistochemical study of eight cases. *Histopathology* 1986; 10: 589-600.
4. Stein H, Dallenbach F. Diffuse large cell lymphomas of B and T cell type. In: Knowless DM, ed. *Neoplastic Hematopathology*. Baltimore: Williams and Wilkins 1992:675-714.
5. Makiguchi N, Ohsaki Y, Fujiuchi S et al. Ki-1 positive anaplastic large cell lymphoma with mediastinal involvement. *Jap J Thoracic Diseases* 1997; 35: 660-664.
6. Lukes RJ, Collins RD. Immunological characteristics of human malignant lymphomas. *Cancer* 1974; 34: 1488-1503.
7. Hausner RI, Rosas-Uribe A, Wickstrum DA et al. Non-Hodgkin's lymphoma in the first two decades of life. *Cancer* 1977; 40: 1533-1547.
8. Nathwani BN, Kim H, Rappaport H. Malignant lymphoma, lymphoblastic. *Cancer* 1976; 38: 964-983.
9. Rosen PJ, Feinstein DI, Pattengale PK et al. Convoluted lymphoblastic lymphoma in adults: a clinicopathologic entity. *Ann Intern Med*. 1978; 89: 319-324.
10. Levitt LJ, Aisenberg AC, Harris NL et al. Primary non-Hodgkin's lymphoma of the mediastinum. *Cancer* 1982; 50: 2486-92.
11. Lichtenstein AK, Levine A, Taylor OR et al. Primary mediastinal lymphoma in adults. *Am J Med* 1980; 68: 509-514.
12. Miller JB, Variakojis D, Bitran JD et al. Diffuse histiocytic lymphoma with sclerosis: a clinicopathologic entity frequently causing superior venacaval obstruction. *Cancer* 1981; 47: 748-56.
13. Trump DL, Mann RB. Diffuse large cell and undifferentiated lymphomas with prominent mediastinal involvement. A poor prognostic subset of patients with non-Hodgkin's lymphoma. *Cancer* 1982; 50: 277-282.
14. Addis BJ, Isaacson PG. Large cell lymphoma of the mediastinum: a B-cell tumor of probable thymic origin. *Histopathology* 1986; 10; 379-390.
15. Möller P, Lammler B, Erberlein-Gonska M et al. Primary mediastinal clear cell lymphoma of B-cell type. *Virchows Arch* (A) 1986; 409: 79-92.
16. Lamarre L, Jacobson JO, Aisenberg AC et al. Primary large cell lymphoma of the mediastinum. A histologic and immunophenotypic study of 29 cases. *Am J Surg Pathol* 1989; 13: 730-739.
17. Lennert K, Feller AC. *Histopathology of non-Hodgkin's lymphomas*. Berlin, Heidelberg, New York: Springer-Verlag; 1990.
18. Jacobson JO, Aisenberg AC, Lammarre L et al. Mediastinal large cell lymphoma. An uncommon subset of adult lymphoma curable with combinated modality therapy. *Cancer* 1988; 62: 1893-1898.
19. Harris NL, Jaffe ES, Stein H et al. A Revised European-American Classification of Lymphoid Neoplasms: a proposal from the International Lymphoma Study Group. *Blood* 1994; 84: 1361-1392.
20. Banks PM, Warnke RA. Mediastinal (thymic) large B-cell lymphoma. In: Jaffe ES, Harris NL, Stein H, Vardiman JW (eds). *World Health Organization Classification of Tumours. Tumours of Haematopoietic and Lymphoid Tissues*. Lyon: IARC Press; 2001, p.175-176.
21. Strollo DC, Rosado de Christenson ML, Jett JR. Primary mediastinal tumors: part II. Tumors of the middle and posterior mediastinum. *Chest* 1997; 112: 1344-1357.

22. Waldron IA, Dohring EJ, Farber LR. Primary large cell lymphomas of the mediastinum: an analysis of 20 cases. *Semin Diagn Pathol* 1985; 2: 281-295.
23. Al.-Sharabati M, Chittal S, Duga-Neulat I et al. Primary anterior mediastinal B-cell lymphoma. A clinicopathologic and immunohistochemical study of 16 cases. *Cancer* 1991, 67; 2579-2587.
24. Cazals-Hatem D, Lepage E, Brice P et al. Primary mediastinal B-cell lymphoma. A clinicopathologic study of 141 cases compared with 916 nonmediastinal large B-cell lymphomas, a GELA (Group d'Etude des Lymphomes de l'Adulte) study. *Am J Surg Pathol* 1996; 20: 877-888.
25. Davis RE, Dorfman RF, Warnke RA. Primary large cell lymphoma of the thymus: a diffuse B-cell neoplasm presenting as primary mediastinal lymphoma. *Hum Pathol* 1990; 21: 1262-1268.
26. Lavabre-Bertrand T, Donadio D, Fegueux N et al. A study of 15 cases of primary mediastinal lymphoma of B-cell type. *Cancer* 1992; 69: 2561--2566
27. Paulli H, Lazzarino M, Gianelli U et al. Primary mediastinal B-cell lymphoma. Update of its clinicopathologic features. *Leukemia and Lymphoma* 1997; 26: 115-123.
28. Todeshini G, Ambrosetti A, Meneghini G et al. Mediastinal large B-cell lymphoma with sclerosis: a clinical study of 21 patients. *J Clin Oncol* 1990; 8: 804-808.
29. Nakagawa A, Nakamura S, Koshikawa T et al. Clinicopathologic study of primary mediastinal non-lymphoblastic non-Hodgkin's lymphomas among the Japanese. *Acta Pathol Japonica* 1993; 43: 44-54.
30. Walewski J. Remission induction in unfavorable histology, non-Hodgkin's lymphoma patients – preliminary comparative evaluation of two chemotherapy programs: CHOP-B and MEVA. *Nowotwory* 1983;33:259.
31. Shipp MA. A predictive model for aggressive NHL. Prognostic Factors in Aggressive Non-Hodgkin's Lymphoma: Who Has „ High Risk" Disease? *Blood* 1994; 83: 1165-1173.
32. Lazzarino M, Orlandi E, Paulli M et al. Treatment outcome and prognostic factors for primary mediastinal (thymic) B-cell lymphoma: a multicenter study of 106 patients. *J Clin Oncol* 1997; 15: 1646-1653.
33. Grogan TM, Kippman SM, Spier CM et al. Independent prognostic significance of a nuclear proliferation antigen in diffuse large cell lymphomas as determined by the monoclonal antibody Ki-67. *Blood* 1988; 71: 1157-1160.
34. Hall PA, Richards MA,Gregory W Petal. The prognostic value of Ki-67 immunostaining in non-Hodgkin's lymphoma. *J Pathol* 1988; 154: 223-229.
35. Miller TP, Grogan TM, Dahlberg S et al. Prognostic significance of the Ki-67-associated proliferative antigen in aggressive non-Hodgkin's lymphoma: a prospective Southwest Oncology Group trial. *Blood* 1994; 83: 1460-1466.
36. Gascoyne R. Prognostic factors in diffuse large B-cell lymphoma. *Revista Espanola de Patologia* 1999; 32: 306-308.
37. Wilson WH, Teruya-Feldstein J, Fest T et al. Relationship of p53, bcl-2 and tumor proliferation to clinical drug resistance in non-Hodgkin's lymphoma. *Blood* 1997; 89: 601-609.
38. Hill ME, MacLennan KA, Cunningham DC et al. Prognostic significance of bcl-2 expression and bcl-2 major breakpoint region rearrangement in diffuse large cell non Hodgkin's lymphoma: a British National Lymphoma Investigation Study. *Blood* 1996; 88: 1046-1051.
39. Rosenberg SA. Validity of the Ann Arbor staging classification for the NHL's. *Cancer Treat Rep* 1977; 61: 1023-1027.
40. Tchen N, Eghbali H, Soubeyran P et al. Mediastinal large B-cell lymphoma. A retrospective anatomic-clinical study of 26 cases. *Bulletin du Cancer* 1997; 84: 704-708.
41. Suster S, Moran CA. Pleomorphic large cell lymphomas of the mediastinum *Am J Pathol* 1996; 20: 224-32.
42. Hughes JH, Katz RL, Fonseca GA et al. Fine-needle aspiration cytology of mediastinal non-Hodgkin's nonlymphoblastic lymphoma. *Cancer* 1998; 84: 26-35.
43. Slagel DD, Powers CN, Melargano MJ et al. Spindle-cell lesions of the mediastinum: diagnosis by fine-needle aspiration biopsy. *Diagnostic Cytopathol* 1997;17(3):167-76.
44. Yu GH, Salhsny KE, Gokaslan ST et al. Thymic epithelial cells as a diagnostic pitfall in the fine-needle aspiration diagnosis of primary mediastinal lymphoma. *Diagn Cytopathol* 1997; 16: 460-465.
45. Scarpa A, Borgato L, Chilosi M et al. Evidence of c-myc gene abnormality in mediastinal large B-cell lymphoma of young adult age. *Blood* 1991; 78: 780-788.
46. Tsang P, Cesarman E, Chadburn A et al. Molecular Characterisation of Primary Mediastinal B-cell Lymphoma. *AJP* 1996; 148: 6, 2017-2025.
47. Copie-Bergman C, Gaulard P, Maouche-Chretien L et al.: The MAL Gene is expressed in primary mediastinal large B-cell lymphoma. *Blood* 1999; 94: 3567-3575.
48. Joos S, Otano-Joos MI, Ziegler S et al. Primary mediastinal (thymic) B-Cell lymphoma is characterized by gains of chromosomal material including 9p and amplification of the REL gene. *Blood* 1996; 87:.1571--1578.

Paper received: 3 January 2002
Accepted: 17 January 2002